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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 39/15, C12N 7/08

A1

(11) International Publication Number: WO 90/01337

(43) International Publication Date: 22 February 1990 (22.02.90)

(21) International Application Number: PCT/GB89/00882

(22) International Filing Date: 3 August 1989 (03.08.89)

(30) Priority data:
8818415.5 3 August 1988 (03.08.88) GB

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(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB, GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: VACCINE

(57) Abstract

A vaccine comprises a non-cytopathogenic strain of bovine viral diarrhoea virus, grown in a bovine derived cell line such as MDBK and killed, for example with β-propiolactone. The adjuvant is Quil A.

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VACCINE

Bovine virus diarrhoea virus (BVDV) is extremely common in cattle in the UK, the remainder of Western Europe, North America, Australia and Africa. Infection with this virus may result in a variety of syndromes and pathologies influenced largely by the age of animals when first infected. In young, previously uninfected calves the virus causes a transient infection. This is associated with leucopenia, and an interrelated period of immunosuppression and increased susceptibility to infection with other microorganisms. BVDV is, after RSV (respiratory syncitial virus), probably the most important virus associated with outbreaks of respiratory disease in young housed calves and because of its immuno-suppressive effect it may be involved in other calf infections, for example enteritis. This virus is also considered to be a major contributor to disease in "feedlot calves" in the USA and Canada. Following recovery, animals exhibit a degree of immunity to reinfection. However, this immunity appears not to be absolute or lifelong.

More serious problems result from infection of pregnant cattle. Abortion may ensue or alternatively deformities may be produced in the foetus that is carried to term; these deformities may result from exposure to virus at the time when immunocompetence is developing and could be the result of an incomplete immune response. Infection of the foetus before immunocompetence develops can result in the foetus remaining viraemic through the period of gestation and the subsequent birth of a calf that remains persistently viraemic, with a non-cytopathogenic form of the virus, and specifically immunotolerant to BVDV for life. Such calves are the animals that die later of mucosal disease; an event triggered by superinfection with a cytopathogenic variant of BVDV.

It has been estimated that about 0.4% of apparently normal beef calves in the UK are viraemic and these animals represent a major source of infection on farms.

Traditionally, viral vaccines fall into two classes: live vaccines containing live viruses which have been treated or grown (attenuated) in such a way as to make them less pathogenic, and vaccines containing killed (inactivated) virus particles. In the context of BVDV, the viruses themselves may be cytopathogenic or non-cytopathogenic. Thus, in principle, four main classes of BVDV vaccine could exist, although the vast majority of commercial vaccines are based on the cytopathogenic virus. Moreover, it is thought by many that live vaccines are unacceptable because live cytopathogenic vaccine strains may produce death from mucosal disease in persistently viraemic animals, and live non-cytopathogenic virus vaccine may infect the foetus in pregnant cattle and result in any of the diseases outlined above.

Infection via the respiratory tract is probably the most important route of transmission of the virus on farms and protection against spread via this route would be expected to have a major beneficial effect in controlling disease due to BVDV.

Parenteral vaccination with inactivated BVDV protected against respiratory infection. In one experiment all of 5 vaccinated calves were resistant to respiratory challenge and all of 5 controls became infected.

The killed BVDV antigens tested induced the production of high titres of neutralising antibodies. These were shown to rise from less than 50 before vaccination to greater than or equal to 2,000- 10,000 units after vaccination.

In the context of a vaccine comprising a killed, non-cytopathogenic virus, we have found that it is advantageous to grow the virus on a cell line which is derived from bovine cells such as the MDBK cell line

(Madin Darby Bovine Kidney; Madin & Darby (1958); available from ECACC, Salisbury, Wiltshire, UK). MDBK cells are available in many laboratories throughout the world. Other bovine cell lines useful in the practice of the invention include EBL cells, NM5 cells, LWC874 cells and CTe cells.

The MDBK cell line is preferably used at passage levels 147-187, more preferably at pass 147 to 157 and most preferably at pass 147. Seed virus is preferably prepared by adding about 10^6 TCD_{50} of BVDV (non-cytopathogenic strain) to confluent cultures of calf testis cells. Calf testis cells are preferred to grow the seed culture because virus yields are higher in these cells, whereas yields of antigen are greater in MDBK cells. The cells may be grown in roller bottles with Eagle's MEM medium and added foetal bovine serum 7.5%, sodium bicarbonate 0.11% and lactalbumin hydrolysate 0.25%. After addition of the virus, the culture may be maintained with 50 ml medium; Eagle's BME with foetal bovine serum 2%, sodium bicarbonate 0.17%, lactalbumin hydrolysate 0.25% and magnesium chloride hexahydrate 0.6%. The culture may be incubated at about 36°C for 5 to 9 days, preferably 7 days, and then subjected to a single cycle of freeze/thaw. The suspension may be centrifuged at about 500g for 4 to 6 minutes, preferably 5 minutes, to remove gross debris and the supernatant fluid stored in small volumes, ready for use, at about -70°C. The titre of the stored seed virus may be determined by assay in cultures of calf testis cells.

Virus antigen is prepared by adding about 1 ml of seed virus, containing about 10^6 TCD_{50} of BVDV, to cultures of MDBK cells. These cells may be grown in roller bottles with Eagle's MEM, foetal bovine serum 10% and sodium bicarbonate 0.11% and are used after about 4 days' growth when the cultures are about 75% confluent. After addition of the virus the culture may be maintained

with 125 ml of BME medium (vide supra). Seven days later when the culture contains about 10^8 cells and a virus titre of about $10^{8.5}$ TCD_{50}, ß-propiolactone is added to a concentration of 1 in 500 and the bottle rolled for 3 hours at 36°C to inactivate the virus. Complete inactivation of the antigen preparation is checked by passage of samples in cultures of calf testis cells. The antigen is stored at -20°C.

Before cell cultures are used for the preparation of seed virus and virus antigen they are checked for the presence of adventitious BVDV. Foetal bovine serum is checked for freedom from virus and BVDV antibody.

One dose of the vaccine is prepared by mixing 1 mg of "Quil A" (Superfos A/S, Denmark) as 50 ul of a stock (20 mg/ml in water) to 4 ml of beta-propiolactone-inactivated virus. This is injected subcutaneously behind the shoulder of calves, aged about 3 months and shown to be free of BVDV antibody, either maternally derived or produced as a result of infection.

Vaccinated calves showed an antibody response (Table 1), determined by ELISA (Howard, Clarke & Brownlie, 1985), which was detected 6 weeks after the first vaccination. These animals and unvaccinated controls were challenged with a strain of BVDV (11249nc) selected because of its tropism for the respiratory tract and consistent rate of naso-pharyngeal shedding. Calves were infected intranasally on week 8. Virus shedding was determined by examination of naso-pharyngeal swabs (blood was also tested) for up to 10 days after challenge and samples were assayed in cultures of calf testis cells. BVDV was recovered from the control animals (Tables 2,3) but not the vaccinated group. The relationship, for individual animals, between antibody levels at the time of challenge and the susceptibility to infection is shown in Table 3.

None of the controls had detectable antibody at the time of challenge and they all became infected and seroconverted (Table 1).

BVDV antigen may be included with other micro-organisms (preferably inactivated) to form a multivalent vaccine. Suitable organisms include respiratory syncytial virus, parainfluenza 3 virus and Mycoplasma bovis.

Instead of using whole virus, it may be advantageous to separate the antigens from the virus and to use them with Quil A and, optionally, suitable carriers and the like. This may be achieved by known means.

Table 1

Antibody responses by ELISA[1] in calves vaccinated with strain Ky1203nc

Group	No. of Calves	week[2] 0	3	6	8	10	12
Non-vaccinated	5	1.4	ND	ND	1.4	2.09 +0.60	2.50 +0.12
Vaccine standard dose	5	1.4	1.4	3.07 +0.30	3.52 +0.13	4.38 +0.25	4.09 +0.29

[1] mean number of units of antibody (10^n) ± SD

[2] calves vaccinated on weeks 0, 3 and 6; challenged on week 8 with strain 11249nc intransally.

Table 2

Effect of vaccination with strain Ky1203nc on infection with BVDV

Group	No. of Calves	No. of calves infected[1] on indicated day 0	4	6	8	10
Non-vaccinated	5	0	1	5	1	0
Vaccine-standard dose	5	0	0	0	0	0

[1] Isolations from nasopharyngeal swabs

Table 3

Relationship between antibody at time of challenge and susceptibility to infection in individual animals

Animal Code No.	Vaccine[1]	Antibody[2]	Virus isolation[3]		Leucopenia %[4]
			N.Ph.swab	Blood	
X502	S	3.71	-	-	5
A21	S	3.57	-	-	0
X694	S	3.51	-	-	0
X657	S	3.42	-	-	0
X684	S	3.38	-	-	5
A10	-	1.4	+	-	46
A407	-	1.4	+	+	48
X192	-	1.4	+	+	53
X658	-	1.4	+	+	52
X659	-	1.4	+	+	54

[1] Animals given standard dose (S), or no vaccine (-)

[2] Units of antibody (10^n) by ELISA on day of challenge, animals arranged in decreasing order

[3] Isolations from nasopharyngeal swab as in Table 2, isolations from blood on day 6

[4] Percentage reduction in cell count, compared to average of 3 preinoculation values

The MDBK (Madin-Darby Bovine Kidney) cell line has been available for about 25 years from the American Type Culture Collection, Rockville, Maryland, USA as ATCC CCL 22. Since 1982, this line has been BVD-free. The same cell line has also been available from the European Collection of Animal Cell Cultures, Porton Down, Salisbury, Wiltshire, UK, as has MDBK from another source, under the accession number ECACC No. 85102401. A sample of the latter deposit has now been deposited with ECACC under the Budapest Treaty, with the date of 2nd August 1989 and the accession number 89080201.

Claims

1. A process for preparing a vaccine comprising the steps of (a) growing non-cytopathogenic bovine viral diarrhoea virus in a cell line derived from bovine cells and (b) admixing virally-derived material thus obtained with Quil A.

2. A process according to Claim 1 wherein the cell line comprises MDBK, EBL, NM5, LWC874 or CTe cells.

3. A process according to Claim 2 wherein the cell line comprises MDBK cells.

4. A process according to any one of the preceding claims wherein, as an initial step, the cell line is inoculated with BVDV grown in calf testis cells.

5. A process according to any one of the preceding claims where the virally-derived material comprises whole viruses.

6. A process according to Claim 5 wherein the virus is inactivated.

7. A vaccine effective against bovine viral diarrhoea virus (BVDV) infections, comprising inactivated non-cytopathogenic BVD and Quil A as an adjuvant therefor.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/00882

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC [5]: A 61 K 39/15, C 12 N 7/08

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC [5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	EP, A, 0109942 (B. MOREIN) 30 May 1984 see page 24, line 25 - page 25, line 9 --	1
Y	US, A, 3838004 (C.A. MEBUS et al.) 24 September 1974 see column 2, line 16 - column 3, line 53 --	1
A	GB, A, 1401565 (THE REGENTS OF THE UNIVERSITY OF NEBRASKA) 30 July 1975 --	
A	US, A, 3839556 (C.A. MEBUS et al.) 1 October 1974 --	
A	US, A, 3869547 (C.A. MEBUS et al.) 4 March 1975 --	
A	US, A, 3293129 (J.A. BAKER) 20 December 1966 --------	

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10th October 1989	- 9. 11. 89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8900882

SA 30378

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 30/10/89
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US-A- 3293129		None	

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